

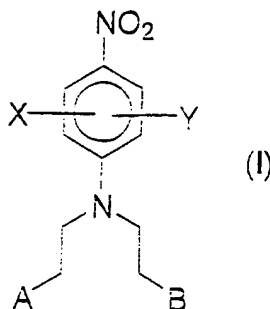
10/529772

JC17 Rec'd PCT/PTO 30 MAR 2005

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

1 (original). A nitroaniline-based unsymmetrical mustard represented by the general formula (I);



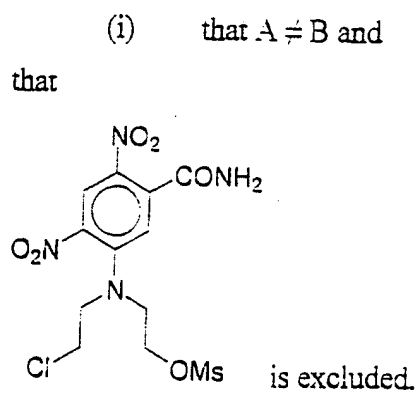
wherein X represents one of the groups NO_2 , CN , or SO_2R^1 , where R^1 represents a C_{1-6} -lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups and wherein when R^1 represents a tertiary amine the N-oxide derivative of the tertiary amine is further included;

Y represents one of the groups OR^2 , NHCOR^2 , $\text{CONR}^2\text{CO}_2\text{R}^3$, $\text{CONR}^2\text{morpholide}$, CONHR^2 , CONR^2R^3 , CONHOR^2 , $\text{CONHSO}_2\text{R}^2$, SO_2NH_2 , SO_2NHR^2 or $\text{SO}_2\text{NR}^2\text{R}^3$ wherein each R^2 and R^3 independently represent a H, C_{1-6} -lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups; and A and B each independently represent halogen, OSO_2R^4 , OSO_2NH_2 , OSO_2NHR^4 or $\text{OSO}_2\text{NR}^4\text{R}^5$, wherein each R^4 and R^5 independently represent a C_{1-6} -lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups and wherein when each R^4 and R^5 independently represents a tertiary amine the N-oxide

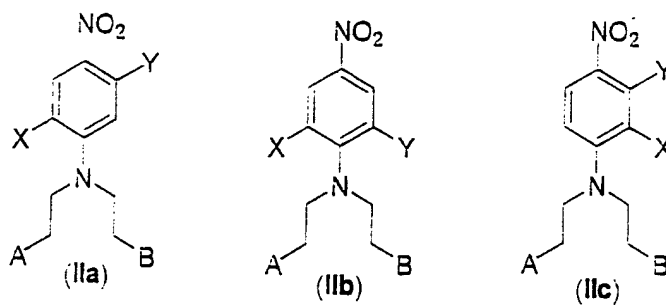
derivative of the tertiary amine is further included;

and pharmaceutically acceptable derivatives and salts thereof;

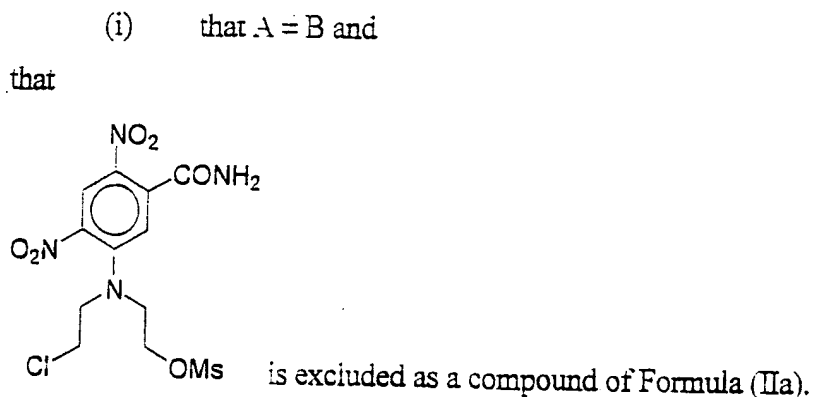
with the proviso



2 (original). The nitroaniline-based unsymmetrical mustard as claimed in claim 1 represented by one of formulae (IIa-IIc)



wherein X, Y, A and B are as defined in claim 1 for a compound of Formula (I);
and pharmaceutically acceptable derivatives and salts thereof;
with the proviso



3 (currently amended). The nitroaniline-based unsymmetrical mustard as
claimed in claim 1 or claim 2 selected from:

- 5-[(2-Bromoethyl)(2-chloroethyl)amino]-2,4-dinitrobenzamide,
- 2-[5-(Aminocarbonyl)(2-bromoethyl)-2,4-dinitroanilino]ethyl methanesulfonate,
- 2-[5-(Aminocarbonyl)(2-iodoethyl)-2,4-dinitroanilino]ethyl methanesulfonate,
- 2-((2-Bromoethyl)5-[(2-hydroxyethyl)amino]carbonyl)-2,4-dinitroanilino)ethyl
methanesulfonate,
- 2-((2-Bromoethyl)5-[(3-hydroxypropyl)amino]carbonyl)-2,4-dinitroanilino)ethyl
methanesulfonate,
- 2-((2-Bromoethyl)-5-[(2,3-dihydroxypropyl)amino]carbonyl)-2,4-
dinitroanilino)ethyl methanesulfonate,

2-[2-(Aminocarbonyl)(2-chloroethyl)-4,6-dinitroanilino]ethyl methanesulfonate,
2[2-(Aminocarbonyl)(2-bromoethyl)-4,6-dinitroanilino]ethyl methanesulfonate,
2-((2-Bromoethyl)-2-[(2-hydroxyethyl)amino]carbonyl)-4,6-dinitroanilino)ethyl
methanesulfonate,

2-((2-Iodoethyl)-2-[(2-hydroxyethyl)amino]carbonyl)-4,6-dinitroanilino)ethyl
methanesulfonate,

2-((2-Bromoethyl)-2-[(2-hydroxypropyl)amino]carbonyl)-4,6-dinitroanilino)ethyl
methanesulfonate,

2-((2-Bromoethyl)-2-[(2,3-dihydroxypropyl)amino]carbonyl)-4,6-
dinitroanilino)ethyl methanesulfonate,

2-[(2-Bromoethyl)-2-({[3-(4-morpholinyl)propyl]amino}carbonyl)-4,6-
dinitroanilino]ethyl methanesulfonate,

Methyl 3-{[2-((2-chloroethyl){2-[(methylsulfonyl)oxy]ethyl}amino)-3,5-
dinitrobenzoyl]amino}propanoate,

Methyl 3-{[2-((2-bromoethyl){2-[(methylsulfonyl)oxy]ethyl}amino)-3,5-
dinitrobenzoyl]amino}propanoate,

2-[3-(Aminocarbonyl)(2-chloroethyl)-2,4-dinitroanilino]ethyl methanesulfonate,
2-[3-(Aminocarbonyl)(2-bromoethyl)-2,6-dinitroanilino]ethyl methanesulfonate,
2-((2-Bromoethyl)-3-[(2-hydroxyethyl)amino]carbonyl)-2,6-dinitroanilino)ethyl
methanesulfonate,

2-((2-Chloroethyl)-3-[(3-hydroxypropyl)amino]carbonyl)-2,4-dinitroanilino)ethyl
methanesulfonate,

2-((2-Bromoethyl)-3-(((3-hydroxypropyl)amino)carbonyl)-2,6-dinitroanilino)ethyl methanesulfonate,

2-((2-Bromoethyl)-3-(((4-hydroxybutyl)amino)carbonyl)-2,6-dinitroanilino)ethyl methanesulfonate,

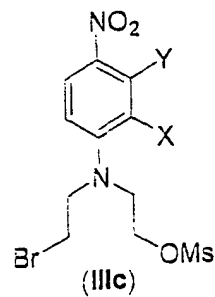
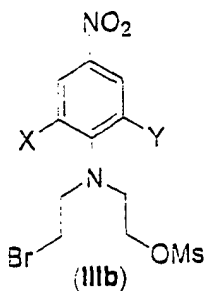
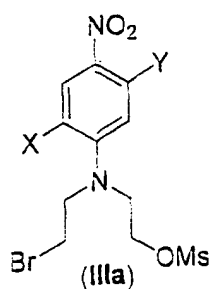
2-((2-Chloroethyl)-3-(((2,3-dihydroxypropyl)amino)carbonyl)-2,4-dinitroanilino)ethyl methanesulfonate,

2-((2-Bromoethyl)-3-(((2,3-dihydroxypropyl)amino)carbonyl)-2,4-dinitroanilino)ethyl methanesulfonate,

2-[(2-Chloroethyl)-3-([3-(4-morpholinyl)propyl]amino)carbonyl]-2,4-dinitroanilino]ethyl methanesulfonate and

2-[(2-Bromoethyl)-3-([3-(4-morpholinyl)propyl]amino)carbonyl]-2,4-dinitroanilino]ethyl methanesulfonate.

4 (currently amended). The nitroaniline-based unsymmetrical mustard as claimed in claim 1 or claim 2 selected from a compound represented by one of formulae (IIIa-IIIc)



wherein X, Y, are as defined in claim 1 for a compound of Formula (I); and
pharmaceutically acceptable derivatives and salts thereof.

5 (original). The nitroaniline-based unsymmetrical mustard as claimed in claim
4 selected from

2-[5-(Aminocarbonyl)(2-bromoethyl)-2,4-dinitroanilino]ethyl methanesulfonate,
2-((2-Bromoethyl)5-[(2-hydroxyethyl)amino]carbonyl)-2,4-dinitroanilino)ethyl
methanesulfonate,

2-((2-Bromoethyl)5-[(3-hydroxypropyl)amino]carbonyl)-2,4-dinitroanilino)ethyl
methanesulfonate,

2-((2-Bromoethyl)-5-[(2,3-dihydroxypropyl)amino]carbonyl)-2,4-
dinitroanilino)ethyl methanesulfonate,

2-[2-(Aminocarbonyl)(2-bromoethyl)-4,6-dinitroanilino]ethyl methanesulfonate,
2-((2-Bromoethyl)-2-[(2-hydroxyethyl)amino]carbonyl)-4,6-dinitroanilino)ethyl
methanesulfonate;

2-((2-Bromoethyl)-2-[(2-hydroxypropyl)amino]carbonyl)-4,6-dinitroanilino)ethyl
methanesulfonate,

2-((2-Bromoethyl)-2-[(2,3-dihydroxypropyl)amino]carbonyl)-4,6-
dinitroanilino)ethyl methanesulfonate,

2-[(2-Bromoethyl)-2-({3-(4-morpholinyl)propyl}amino)carbonyl]-4,6-
dinitroanilino]ethyl methanesulfonate,

Methyl 3-[[2-((2-bromoethyl){2-[(methylsulfonyl)oxy]ethyl}amino)-3,5-

dinitrobenzoyl]amino}propanoate,

2-[3-(Aminocarbonyl)(2-bromoethyl)-2,6-dinitroanilino]ethyl methanesulfonate,

2-((2-Bromoethyl)-3-[[2-(2-hydroxyethyl)amino]carbonyl]-2,6-dinitroanilino)ethyl
methanesulfonate,

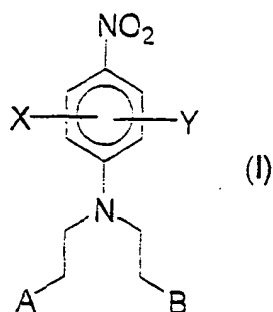
2-((2-Bromoethyl)-3-[[3-(3-hydroxypropyl)amino]carbonyl]-2,6-dinitroanilino)ethyl
methanesulfonate,

2-((2-Bromoethyl)-3-[[4-(4-hydroxybutyl)amino]carbonyl]-2,6-dinitroanilino)ethyl
methanesulfonate,

2-((2-Bromoethyl)-3-[[2,3-dihydroxypropyl]amino]carbonyl)-2,4-
dinitroanilino)ethyl methanesulfonate and

2-[(2-Bromoethyl)-3-((3-(4-morpholinyl)propyl)amino)carbonyl]-2,4-
dinitroanilino]ethyl methanesulfonate.

6 (original). A method of preparing a nitroaniline-based unsymmetrical mustard
represented by the general formula (I);



wherein X represents one of the groups NO₂, CN, or SO₂R¹, where R¹ represents a C₁₋₆-lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups and wherein when R¹ represents a tertiary amine the N-oxide derivative of the tertiary amine is further included;

Y represents one of the groups OR², NHCOR², CONR²CO₂R³, CONR²morpholide, CONHR², CONR²R³, CONHOR², CONHSO₂R², SO₂NH₂, SO₂NHR² or SO₂NR²R³ wherein each R² and R³ independently represent a H, C₁₋₆-lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups; and A and B each independently represent halogen, OSO₂R⁴, OSO₂NH₂, OSO₂NHR⁴ or OSO₂NR⁴R⁵, wherein each R⁴ and R⁵ independently represent a C₁₋₆-lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups and wherein when each R⁴ and R⁵ independently represents a tertiary amine the N-oxide derivative of the tertiary amine is further included;

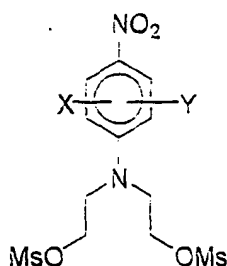
and pharmaceutically acceptable derivatives and salts thereof;

with the proviso

(i) that A ≠ B

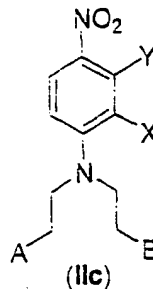
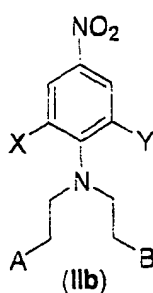
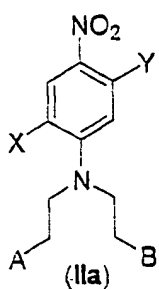
the method including the step of

reacting a compound of



with an amount of an alkali metal halide in a polar solvent to give an unsymmetrical halo-mesylate compound.

7 (currently amended). The method of preparing a nitroaniline-based unsymmetrical mustard represented by the general formula represented by one of formulae (IIa-IIc) as claimed in claim 2 ~~or claim 3~~

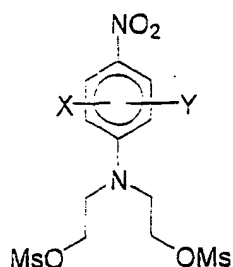


wherein X, Y, A and B are as defined in claim 1 for a compound of Formula (I);
and pharmaceutically acceptable derivatives and salts thereof;

with the proviso

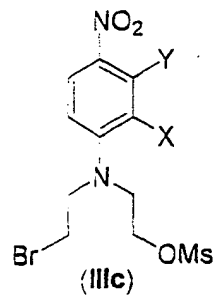
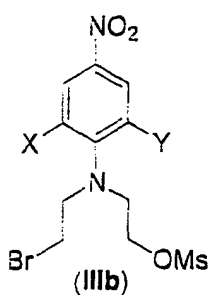
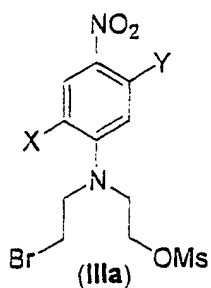
(i) that $A \neq B$ and

the method including the step of
reacting a compound of



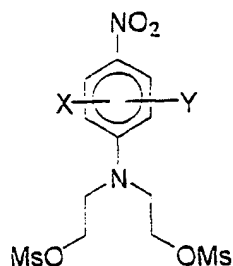
with an amount of an alkali metal halide or mesylate halide in a polar solvent to give an unsymmetrical halo-mesylate compound.

8 (currently amended). The method of preparing a nitroaniline-based unsymmetrical mustard represented by one of formulae (IIIa-IIIc) as claimed in claim 4 or claim 5



wherein X, Y, are as defined in claim 1 for a compound of Formula (I); and pharmaceutically acceptable derivatives and salts thereof; the method including the step of

reacting a compound of



with an amount of LiBr in a polar solvent to give a bromo mesylate of one of formulae (IIIa-IIIc).

9 (currently amended). The method as claimed in ~~any one of claims 6 to 8~~ claim 6 wherein the polar solvent is selected from acetonitrile, dimethylformamide, ethyl acetate, triethylamine, acetone and mixtures thereof.

10 (currently amended). The method as claimed in ~~any one of claims 6 to 9~~ claim 6 wherein the alkali metal halide is selected from one or more of the following; LiCl, LiBr, NaI and NaBr.

11 (currently amended). A compound of formula (I) obtained by any one of the methods as claimed in ~~any one of claims 6 to 10~~ claim 6.

12 (currently amended). A method including the step of administering a compound of Formula I as defined in ~~any one of claims 1 to 5~~ claim 1 in a

therapeutically effective amount to tumour cells in a subject for the use as prodrugs suitable for GDEPT (gene-dependent enzyme-prodrug therapy) in conjunction with at least one nitroreductase enzyme, as a hypoxia-selective cytotoxin.

13 (original). The method according to claim 12 wherein the nitroreductase enzyme is encoded for by the *nfsB* gene of either *E. Coli* or by *Clostridia* species.

14 (currently amended). A method including the step of administering a compound of Formula I as defined in claim 1 in a therapeutically effective amount to target tumour cells in a subject, for the use as prodrugs suitable for GDEPT (gene-dependent enzyme-prodrug therapy) in conjunction with at least one nitroreductase enzyme, as an anticancer agent.

15 (original). The method according to claim 14 wherein the nitroreductase enzyme is encoded for by the *nfsB* gene of either *E. Coli* or by *Clostridia* species.

16 (original). A method of cell ablation therapy utilising at least one nitroreductase enzyme, wherein the method includes the step of administering a compound of Formula I as claimed in claim 1 in a "therapeutically effective amount" to ablate tumour cells in tissue in a subject, wherein said tissue expresses at least one nitroreductase enzyme.

17 (original). The method according to claim 16 wherein the nitroreductase

enzyme is encoded for by the *nfsB* gene of either *E. Coli* or by *Clostridia* species.

18 (currently amended). The method according to claim 16 ~~or claim 17~~
wherein the cell ablation therapy provides a substantially minimal bystander effect.

19 (original). A pharmaceutical composition including a therapeutically effective amount of a compound of formula I as defined in claim 1 and a pharmaceutically acceptable excipient, adjuvant, carrier, buffer or stabiliser.

20 (original). The use in the manufacture of a medicament of an effective amount of a compound of Formula I as defined in claim 1 for use in GDEPT to target cancer cells in a subject in need thereof.

21 (original). The use in the manufacture of a medicament of an effective amount of a compound of Formula I as defined in claim 1 for use in cell ablation therapy to target cancer cells in a subject in need thereof.